THE EFFECT OF TEMPERATURE CHANGES UPON THE PULSATIONS OF ISOLATED SCALE MELANOPHORES OF *FUNDULUS HETEROCLITUS*

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When Spaeth (1916*a*) published a method for producing pulsations in the isolated scale melanophores of *Fundulus heteroclitus* he greatly broadened the possible scope of investigations dealing with the direct action of various physical and chemical factors upon such preparations. Recognizing this fact, it was thought that perhaps an investigation of the effects of temperature changes upon such pulsations would help in extending our knowledge of how heat and cold act upon the pigment cells. The melanophores of isolated *Fundulus* scales will respond directly to variations in temperature (Spaeth, 1913; Smith, 1928), heat causing a contraction and cold an expansion of the pigment granules. But beyond these descriptive facts our knowledge does not go. One's interest is, therefore, directed towards ascertaining how these melanophores, when induced to pulsate through the use of Spaeth's method, would react to temperature changes, with the hope of perhaps obtaining information permitting a more exact analysis of this phenomenon.

Briefly, Spaeth's method of producing pulsations is as follows: First the isolated *Fundulus* scales are immersed in N/10 $BaCl_2$ for 5 minutes, in which time their melanophores become punctate; then they are transferred to N/10 NaCl, where within fifteen to twenty minutes after their removal to this solution, the pulsations become evident. These pulsations take the form of periodic migrations of the pigment granules in and out of the processes of the melanophores, the movements of the individual pigment granules being readily seen with ordinary microscopic magnification. Hence, we have a preparation which, in its rhythmical activity, is comparable to the behavior seen in other effector organs such as cilia, heart muscle, and smooth muscle. To this add the advantage that in the melanophore we deal with an effector consisting of only a single cell. In studying the effects of temperature changes upon pulsating pigment cells, our interest is therefore centered on how this factor may alter either the rate or the extent of these rhythmical migrations, distal and proximal, of the pigment.

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Now it is well known that the color changes exhibited by numerous fishes in response to variations in the tint of the background over which they swim are determined in a large measure by the behavior of these melanophores and allied pigment cells. However, in the intact fish the melanophores do not pulsate; such alterations as take place in the distribution of their pigment are relatively slow in occurrence and only in response to a change in the color of the background or of the incident light intensity. Therefore, in dealing with pulsating isolated melanophores, we are not concerned with a phenomenon which plays a part in the natural economy of the animal.

As a cell the melanophore is a relatively large structure with numerous branching processes exceeding in length by three or four times the diameter of the central body from which they ramify. These processes, when empty of pigment, are transparent under ordinary conditions of illumination. In fact, the visible manifestation of the shape of the cell is due entirely to the pigment granules contained within itself, and if this pigment is concentrated in the central body, the cell appears to be spherical. The term punctate is applied to melanophores in this state. But when under the proper circumstances these invisible processes are invaded by a distal migration of the pigment granules, their outlines are rendered visible. It is this migration of the pigment in and out of the otherwise transparent processes that creates the illusion of a change in the shape of the melanophore. The use of the terms "contraction" and "expansion" of the melanophores must, therefore, be considered as only convenient expressions describing a redistribution of the pigment granules within the melanophores.

An investigation of this nature requires an apparatus sensitive enough to accurately measure these pulsatory movements within the processes of the melanophores. Such an apparatus was modeled with some modifications after one originally devised by Spaeth (1916*b*) for the same purpose, and employed by him in connection with his researches upon the effects of electrical stimulation upon the isolated melanophore. As my investigations were not concerned with the effects of electrical stimulation, but were rather studies of the effects of temperature variations, certain changes were necessarily introduced in the original design, which is fully described and pictured elsewhere (Spaeth, 1916*c*); the most important among these changes being the addition of a warm stage to the set-up. This was done by fitting to the stage of the microscope a water bath in which a small Stender dish could be almost completely submerged. This Stender dish was filled with N/10 NaCl, and in this solution the scales containing the pulsating melanophores were placed. By altering the temperature of the water

flowing through the bath, it was also possible to change the temperature of the NaCl. Any desired temperature between 0° and 45° C. could be attained within two minutes in the Stender dish and maintained indefinitely with no variation greater than 0.2° C.

To measure the pulsations and to make a permanent record of their variations, the microscope was equipped with an adjustable micrometer ocular in place of the usual eye piece, the adjusting screw of the ocular being fitted to a pulley operating a lever capable of writing upon a kymograph drum. In making a record, a scale was placed under the objective, and one of its pulsating melanophores selected for observation, a convenient division of the ocular scale being brought level and at right angles to the apparent end of one of the pulsating processes of this selected melanophore. As the pigment granules in this process migrated either distally or proximally, the ocular scale division kept pace with those granules which marked the apparent distal boundary of the process. Such action was accomplished by turning the adjusting screw of the movable ocular scale. To insure accurate measurements, it was absolutely necessary that the melanophore be kept motionless throughout the experiment. This was accomplished first by bringing a microscope clamp, attached to the warm stage, to bear lightly upon the end of the scale, care being taken that the melanophores were not damaged or stimulated by too great a pressure. Since such a contingency was always imminent, this method for holding the scale was eventually discarded in favor of another. The new method consisted in laying a paraffine mould on the bottom of the Stender dish cut so as to accommodate the scale. In this way the scale was snugly secured without being subjected to any noticeable pressure. No effect of the paraffine upon the pulsations could be demonstrated. As the adjusting screw was turned to follow the migrations of the distal pigment granules, the pulley attached to it moved the lever in contact with the kymograph drum up and down. Consequently, with the lever writing upon the smoked kymograph paper, a permanent and accurate record of the pulsations could be obtained. The speed of the drum was adjusted to one revolution in approximately forty-five minutes.

Records of the melanophore pulsations were not made until the cells had been exposed to NaCl for at least one hour subsequent to their immersion in $BaCl_2$. While pulsations are first evident in fifteen to twenty minutes after the removal of the scales from $BaCl_2$, it usually requires an additional thirty minutes before they become constant in extent and frequency at any given temperature. The first pulsations are extremely slight, but once started they gradually become more pronounced until they finally reach the maximum characteristic for

the particular temperature in question. An hour, then, gives ample time to insure the attainment of this point. However, once this maximum was reached, a new state of equilibrium incident upon a change in temperature could be attained within 5 minutes. Therefore, a record having been made at a certain temperature, 10 minutes being usually sufficient for this purpose, another record at another temperature could be made 10 minutes after the change in temperature was effected. The extent of the temperature change between observations was from 1° to 10° C., the usual variation being about 5° C. The experiments were begun at either end of the temperature scale, and by a series of four or five steps, the temperature was raised or lowered to the opposite extreme, the result being the same regardless of whether the initial record was made at a high or a low temperature. In this way one melanophore was kept under continuous observation for two or three hours. At the beginning, a single process of a single melanophore was selected, and the record made from the pulsations of this process. The numerous melanophores of a single isolated scale vary considerably in size. Though an attempt was made to confine the experiments to melanophores approximately equal in size, this was not always possible. However, such attempts were more or less nullified by the fact that it was impossible to predict the maximal length of a process by its appearance when the cell was in an almost punctate or even semi-expanded condition. As fully one-half the experiments were begun under such circumstances, certain corrections in the data were necessary before the results could be subjected to satisfactory analysis.

Temperature Changes and their Effect upon the Extent of the Pulsations

On analysing the results obtained from the records pertaining to the relationship between the temperature and the amount of extension of the melanophore processes during their pulsations, two general facts emerge: (1) An increase in temperature causes a decrease in the extent of the pulsations and (2) this relationship between temperature and extension is a rectilinear one. Table I presents the data relative to this relationship. The figures given in the temperature columns represent the average of the mean maximal and minimal lengths of a single selected melanophore process during its pulsations at a given temperature, obtained by measuring in centimeters the height of the highest and lowest points of the curve traced on the kymograph records. This averaging was necessary for two reasons: first because during the pulsations the maximal extensions of a single process were not

Table I

Average Migration

C.	9.5°	12°	13°	13.5°	14°	14.5°	15°	15.5°	16°	16.5°	17°	17.5°	18°	18.5°	19°	19.5°	20°	20.5°	21°	21.5°	22°
1				11.5												8					
2							38.5					34.5			30						
3	38	38					32		25.5		13.5										
4											34					25					
5																		16			
6												32									
7																					
8											28						25				
9																					
10																				27	
11									11								7				
12							25.5								18.5						
13			34										23								
14							25										9				
15									37												
16									18.5											11	
17								21									18.5				
Av.							30.2		23		25						15.0				

TABLE I (*Continued*)

Average Migration

C.	22.5°	23°	23.5°	24°	24.5°	25°	25.5°	26°	26.5°	27°	27.5°	28°	28.5°	29°	29.5°	30°	30.5°	31°	31.5°	32°
1							4								1					
2	16.5							10												
3																				
4	17				12															
5	13					11.5					6.5									
6					23								8.5						1.5	
7	39			27				17				6.5								0
8		17.5						6.5						1						
9	23			18			18				8				5			1.5		
10				23					18							8 5				
11		9										2								
12		14.5						10						6						
13	14						12								8					
14	6.5							4.5												
15						19		12			8.5									
16						5.5										1				
17				13					3.5					.5						
Av.	18.4	14.0		20.0		12.0	11.3	10.0			7.7			2.5	4.7					

always constant at a given temperature, a fact which applies equally to the minimal extensions, and secondly because at a point two or three degrees above the lowest temperature at which pulsations occurred (about 12° C.), the process would attain during its period of greatest extension its maximal possible length. However, over these same low temperatures the length of the process during the phase of minimal extension was greater, the lower the temperature, the minimal extension gradually approaching the maximal extension as the temperature came nearer to the critical point. Similarily, as the temperature rose to within two or three degrees of the upper limit (about 32° C.) at which pulsations stopped, the melanophores would appear punctate during their phases of minimal contraction, though at these temperatures the process would keep on periodically extending; the amount of extension, however, decreasing as the temperature rose. On reaching the upper limit all pulsations ceased and the cells remained permanently punctate. Therefore, by using averages of the maximal and minimal lengths, figures are obtained which at the extreme temperatures vary in accordance with the temperature changes. This, of course, necessitates treating the data obtained in the middle of the temperature scale in the same manner.

As indicated in Table I, whenever observations were made upon three or more different processes at the same temperatures, an average of the figures expressing the relative amount of extension was made. These averages are given in the bottom line of the table and are plotted with the result shown in Fig. 1. From this curve it is clear that as the temperature increases, the extension of the melanophore processes during their pulsations decreases; a result to be expected, since it was previously known that high temperatures, when they affect the melanophores directly, cause a withdrawal of the pigment into the central body. Furthermore, it is quite clear from Fig. 1 that the relationship between temperature and extension is a rectilinear one.

The observational data given in Table I represent the true relative lengths of the melanophore processes as they were calculated from the kymograph records. It is apparent, from an inspection of the table, that there is considerable variation in the pulsatory activity among the different processes in regard to the amount of extension shown at the same temperature. Because of this variation, the figures cannot be profitably compared unless they are all corrected on the assumption that the amount of extension in any one process at 20° C. is equal to twenty. When this is done and the results plotted as shown in Fig. 2, it is obvious that the effect of temperature changes on all pulsating melanophore processes is virtually the same so far as any relative alter-

ation in the amount of extension is concerned. The one exception is case 3 of Table I, where the corrected figures were so at variance with the others that it was impossible to plot them upon the curve. They have consequently been omitted from Fig. 2. But aside from

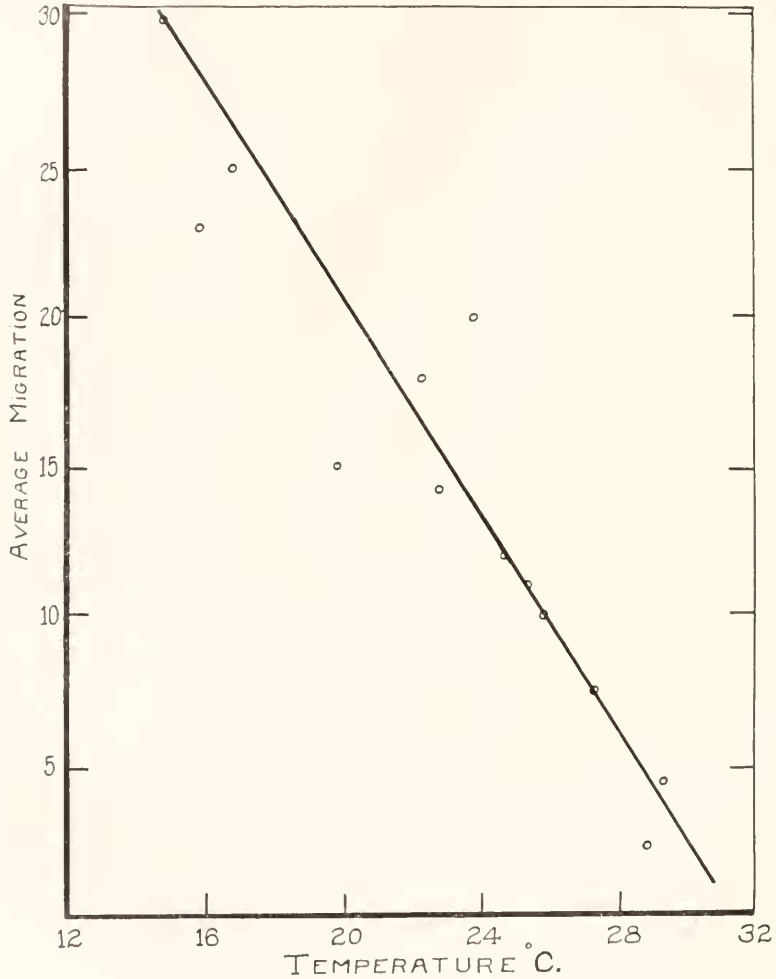

FIG. 1. Showing the rectilinear relationship between the temperature of the medium (abscissæ) and the average length of a melanophore process during a single pulsation (ordinates). The points plotted are those given in the average column of Table I.

this unexplained discrepancy, the curve substantiates the conclusions drawn from the one given in Fig. 1.

In the higher reaches of the temperature scale pulsations cease

between 30° and 34° C., the average being approximately 32° C. When this occurs, the melanophore assumes a permanent punctate state. At low temperatures, however, the melanophore comes to rest with all of its processes maximally extended, the pigment being evenly, if thinly, distributed throughout the whole length of the process. Such a condition is generally attained at about 12° C., slight move-

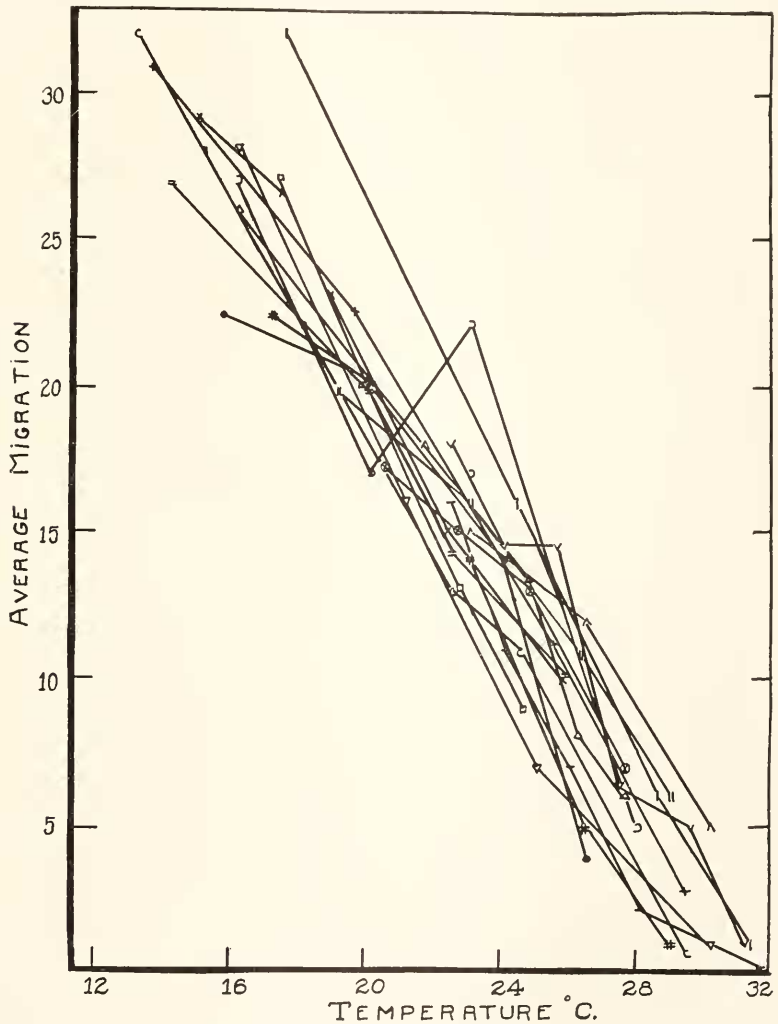

FIG. 2. Showing the result of plotting seventeen different experiments on the relationship between the temperature of the medium (abscissæ) and the average length of the melanophore process during a single pulsation (ordinates); the average length of the melanophore process being taken in all cases as equal to 20 at 20° C., the data in Table I being corrected accordingly.

Table II

Pulsations per Minute

C.	9.5°	12°	13°	13.5°	14°	14.5°	15°	15.5°	16°	16.5°	17°	17.5°	18°	18.5°	19°	19.5°	20°	20.5°	21°	21.5°	22°
1				.6												1.8					
2							.7					.52			.85						
3	.5						.9		1.1		1.35										
4											.8					1.2					
5																		.4			
6												.4									
7																					
8											.8						.9				
9																					
10																				1.3	
11									.8								1.2				
12							.8								1.5						
13			.2										.4								
14							.4										.9				
15									.8												
16									1.3											1.8	
17								.3									.4				
Av.							.7		1.0		.98						.85				

TABLE II (*Continued*)

Pulsations per Minute

C.	22.5°	23°	23.5°	24°	24.5°	25°	25.5°	26°	26.5°	27°	27.5°	28°	28.5°	29°	29.5°	30°	30.5°	31°	31.5°	32°
1							2.5								3.0					
2	1.4							2.0												
3																				
4	1.9				2.1															
5	.7					1.1					1.8									
6					.6								1.7						1.1	
7	1.1			1.3				1.7				2.4								0
8		1.0						1.5						1.2						
9	1.2			2.1			1.8				2.1				2.2			1.8		
10				1.6					1.8							2.3				
11		2.1										5.0								
12		4.0						4.4						1.1						
13	.9						1.3								2.4					
14	1.2							2.4												
15						2.0		2.8			3.4									
16						3.7										8.0				
17				1.0					1.4					.8						
Av.	1.2	2.3		1.5		2.27	1.87	2.46			2.43			1.03	2.53					

ments persisting in some cells until 10° C.; while others come to rest at temperatures as high as 14° C. In many cases Brownian movement was observed among the pigment granules in all parts of the process, even though the pulsations had entirely ceased.

Temperature Changes and their Effect upon the Frequency of Pulsations

On considering the action of temperature changes upon the frequency of pulsations, we find (1) that an increase in temperature from 12° C. to within the neighborhood of 27° C. causes an increase in the rate of pulsations, which is followed by a rapid decrease as the temperature mounts higher, and (2) that the relationship between temperature and the increase in rate during the rise from 12° to about 27° C. is a non-rectilinear one. The data obtained from an analysis of the kyomograph records are presented in Table II, where the figures given in the columns under the temperature headings show the absolute number of pulsations of a single melanophore process in one minute at the temperature indicated. Every figure in the temperature columns represents the pulsations per minute of a different process, no two processes being attached to the same melanophore and every melanophore being selected from a different scale. Between different cells the variation in the frequency of the pulsations at the same temperature is considerable, as shown in Table II; at 22.5° C., for example, a process of one cell shows 0.7 pulsation per minute, while a process of another cell shows 1.9 pulsations per minute. In every case where three or more observations were made at the same temperature, the results were averaged, the averages being given in the bottom line of the table.

In Fig. 3 these averages are plotted, such plotting bringing out the previously-mentioned increase in rate of pulsation during the greater part of the temperature rise, the relationship in this case, in contrast to the relationship between temperature changes and the extent of the pulsations, being clearly non-rectilinear in nature. The possible significance of this will be discussed later. From this curve the temperature coefficient of the melanophore pulsations can be calculated, though such coefficients can have only an approximate value. Between 15° C. and 25° C. the temperature coefficient is 3.57, while between 20° C. and 30° C. it is 3.6. It is doubtful whether the difference between the two is significant, especially as the latter coefficient can have only a speculative value, owing to the irregular appearance of the depressant effect upon the rate of pulsations at the warmer end of the temperature scale.

As there is a wide variation in the behavior of different processes at the same temperature, the data presented in Table II must be corrected before they can be presented in intelligible graphic form. In doing this it was arbitrarily assumed that the rate of pulsation of all melanophore processes at 20° C. was equal to one, the necessary corrections for all other temperatures being made on this basis. These

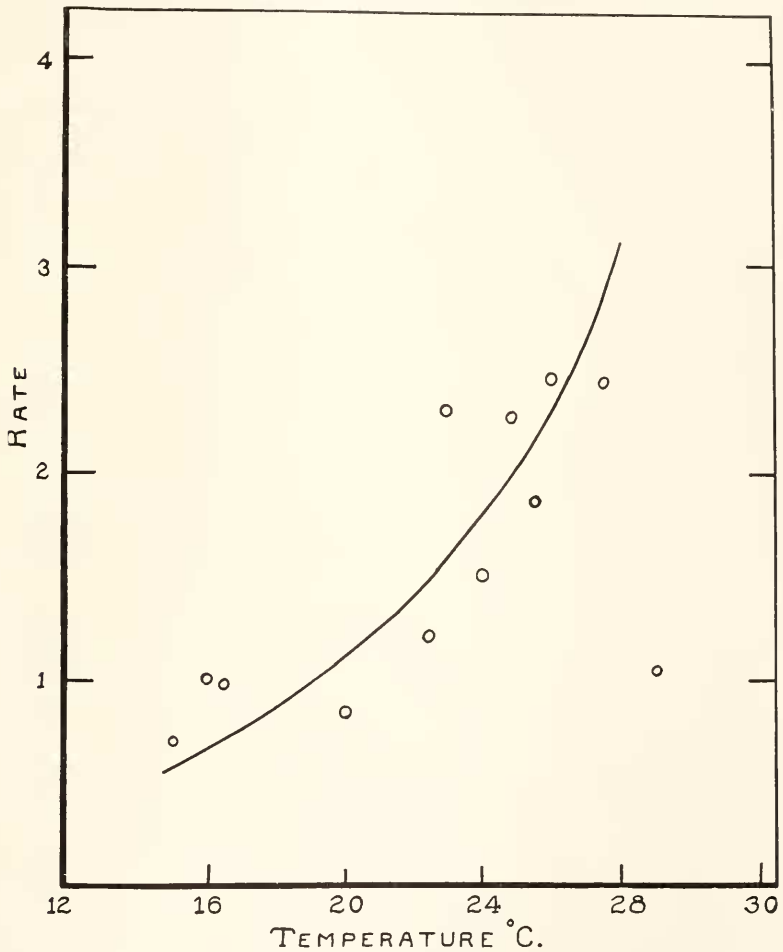

FIG. 3. Showing the non-rectilinear relationship between the temperature of the medium (abscissæ) and the number of pulsations per minute (ordinates). The points plotted are those given in the average column of Table II.

corrected data were then plotted as shown in Fig. 4, the curve so obtained substantiating the relationship expressed in Fig. 3. Furthermore, the points of Fig. 4 group themselves closely about the curve given in Fig. 3.

As to the point where the frequency of pulsations decreases with further increases in temperature, there is considerable variation among the different melanophores, some showing no diminution in the in-

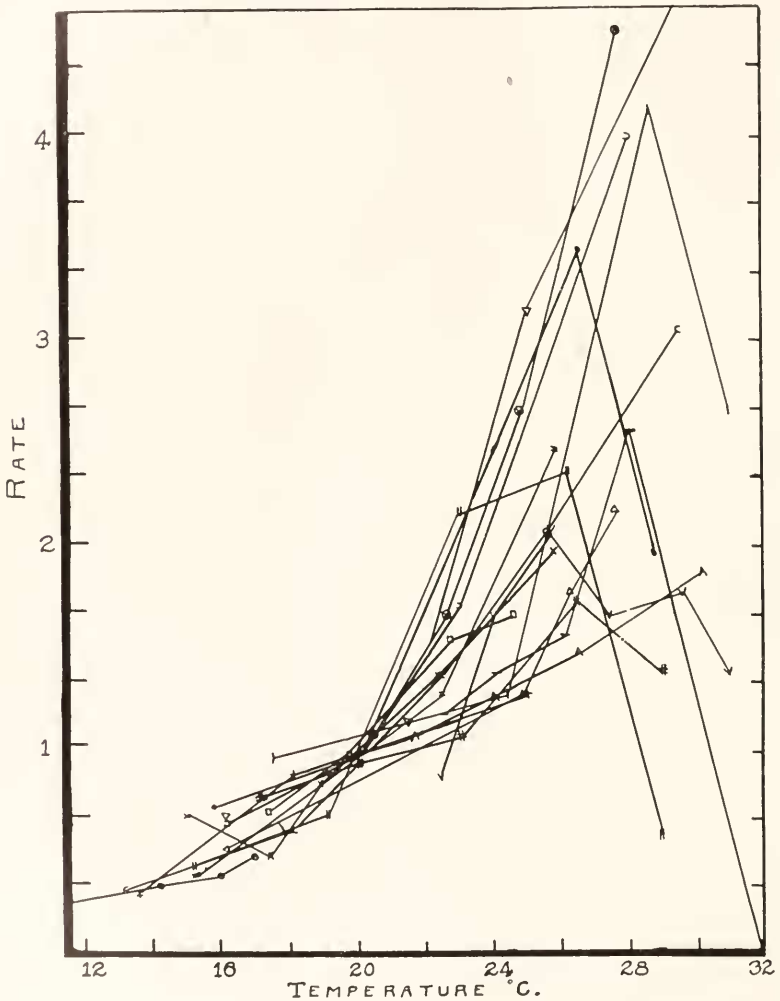

FIG. 4. Showing the result of plotting seventeen different experiments on the relationship between the temperature of the medium (abscissæ) and the number of pulsations per minute (ordinates); the number of pulsations per minute being taken in all cases as equal to one at 20° C., and the data in Table II corrected accordingly.

crease in rate at temperatures as high as 30° C., while in others this depressant effect is initiated at temperatures as low as 25° C. In general, this point occurs in the neighborhood of 27° C. Once a

decrement in rate is established, a further rise in temperature produces a fall, which is very rapid in comparison to the relatively slow increment which preceded it, the difference being graphically shown in Fig. 4. The exact course of this reduction in rate is uncertain, as the data at hand do not permit an accurate analysis.

Discussion

A rise in temperature, then, affects the pulsating pigment granules of an isolated *Fundulus* scale melanophore by increasing the frequency of their pulsatory movements up to a certain point and at the same time reducing the distance migrated by these granules during their pulsations, which in effect gives the appearance of a shortening of the melanophore process. Gray (1923) mentioned a somewhat similar situation in his study of the action of temperature changes upon ciliary activity when he observed a progressive reduction in the amplitude of the ciliary beat between 34° and 38° C., though the frequency of the beat increased in proportion to the rise in temperature. In Gray's experiments, however, this reduction in amplitude was seen only above 34° C. Between 0° and 34° C. the amplitude of the beat remained constant, though the frequency increased as the temperature became higher. One must, therefore, make a distinction between cilia and melanophores in this respect, for in the latter any temperature rise capable of increasing the frequency of the pulsation also produces a corresponding decrease in the extension of the granules during these pulsations. Gray suggests that in his experiments the decrease in the amplitude of the ciliary beat above 34° C. might be explained by assuming "that at this temperature the rate at which the process of contraction is induced in the cilium is more rapid than the process of relaxation, so that relaxation begins before contraction is complete."

The decrease in apparent extension of the melanophore process with an increase in temperature might also be explained in the same way. Once a distal migration of the pigment granules has been established, there is the possibility of its continuing until either factors are brought into play, producing a movement in the opposite direction, or until the granules reach the end of the process and are incapable of further migration. But except with extreme low temperatures, proximal pigment migration sets in before the limits of distal migration are reached. Therefore, the factors producing this proximal migration are capable of overcoming those governing the distal migration, or else the latter cease to operate before the pigment granules reach the limits of the process. But whichever explanation is correct, the matter is of secondary importance, as the factors determining the extent of

the migration of the pigment granules must be independent of both. This conclusion is deducible from the response of melanophore pulsations to variations in temperature, for such variations seem to influence independently two separate series of reactions within the pigment cell, one governing frequency, and the other the extent of the migration. The fact that the distance migrated by the pigment granules during their pulsations shows a rectilinear relationship while the frequency of the pulsations varies in a non-rectilinear manner in relation to temperature changes hardly warrants any other interpretation. Therefore, if extension is determined by frequency, and it is by no means clear that this is so, it is only because the factors leading to a proximal migration dominate those which might favor further extension and not because extension is a function of frequency. It is interesting to note in this connection that Clark (1920) demonstrated in the isolated rabbit's auricle a rectilinear decrease in the height of contraction with an increase in temperature; and at the same time observed a non-rectilinear increase in the frequency of the beat. In isolated frog's heart this was not the case, both the diminution in the force of contraction and the increase in frequency of the beat caused by a rise in temperature being of a non-rectilinear nature.

Since melanophores contract in the presence of an insufficient amount of oxygen, there may exist a relationship between the decrease in the oxygen tension of the water with an increasing temperature and the decrease in extension of the melanophore processes. But Spaeth (1913) has shown that in the isolated scale, melanophore contraction of the pigment occurs at high temperatures both in media furnished with an excess supply of oxygen and in media with the usual oxygen tension for the temperature in question. Therefore, the contraction of the pigment granules with an increase in temperature cannot be due to a lack of oxygen. But the contraction of pigment in a non-pulsating melanophore may be governed by factors other than those which control the amount of extension of the pigment granules in a pulsating melanophore. Unfortunately this question is left open as my observations did not include the effect of a decrease in oxygen supply upon either the frequency or the extent of pulsations and nothing is known concerning this question from other sources. The sudden decrease in the frequency of pulsations with temperatures above 27° C., following a consistent increase with a rise in temperature to this point, may be concerned with a possible inadequacy in the oxygen supply to the melanophores at high temperatures. Whether or not this is true for pulsating melanophores, it is apparently untrue in the case of cilia, for, according to Gray (1923) the oxygen consumption of the tissue

keeps pace with the speed of the beat over the temperatures used in his experiment. As a consequence of this, Gray doubts whether a rise in temperature involves in well-ventilated tissues a lack of oxygen except with very high temperatures, when a decrease in mechanical activity might well be due to insufficient oxygen.

The effects of temperature changes upon pulsating melanophores and the effects of temperature changes upon ameboid movement, ciliary activity, and heart beat show a certain similarity. In all cases, the effect is one of an increase in frequency, an increase which is more rapid than a rectilinear function. But while the temperature coefficient of ameboid movement, ciliary activity, and heart beat are all in fairly close agreement, the melanophore is peculiar in that it has a temperature coefficient which in comparison to the others is remarkably high. As we have previously seen, the temperature coefficient of a pulsating melanophore is 3.57 between 15° and 25° C., and 3.6 between 20° and 30° C., figures which can be compared with those given in Table III.

TABLE III

Q_{10}	15°–25° C.	20°–30° C.
Ciliary activity	2.15	1.95 (Gray, 1923)
Ameboid movement	2.04	— (Pantin, 1924*b*)
Heart beat	2.10	1.90 (Clark, 1920)
Melanophore pulsations	3.57	3.60

A determinative rôle on the part of viscosity changes has already been assigned as a possible factor controlling the movements of pigment granules within the melanophores and it is easy to imagine the same factor assuming considerable importance in governing the frequency and the extent of melanophore pulsations. But unfortunately our knowledge of the exact effect of temperature changes upon the viscosity of protoplasm is at present too meager and too contradictory to warrant any extended conclusions as to how much viscosity is involved in responses of melanophore pulsations to variations in temperature. Both Pantin (1924*a*) and Heilbrunn (1924) have interested themselves in the relationship between temperature and its effect upon animal protoplasm, but with no agreement as to results. Pantin, who worked with the protoplasm of *Nereis* eggs, states that as the temperature increases, the viscosity decreases, the relationship being a non-rectilinear one with a temperature coefficient of 1.3 between 15° C. and 25° C. and 1.26 between 20° C. and 30° C. Heilbrunn, on the other hand, working with *Cumingia* eggs found a sharp decrease in viscosity between 0° C. and 2° C., followed, as the temperature rose further, by a gradual increase which came to a maximum in the neighborhood of

16° C. This in turn was replaced by a decrease until the temperature reached about 30° C., when within the next degree or two there was a very rapid increase. But on the basis of either Pantin's or Heilbrunn's work it is clear that viscosity is not an important factor in determining the responses of melanophore pulsations to temperature changes. The difference between the temperature coefficients which Pantin obtained and those found to apply to melanophore pulsations are so great that the viscosity changes in the melanophores can hardly be of importance in governing the variations in pulsations associated with temperature changes. While, on the basis of Heilbrunn's results, if viscosity changes within the melanophore were an important factor, the curves obtained in these studies on the relationship between temperature changes and the amount of extension of the pigment granules together with the frequency of their pulsations would hardly be as regular as they are, but would show significant variations or breaks in accordance with the curve obtained by Heilbrunn. For the present at least, the conclusion seems inevitable that viscosity changes in the protoplasm of the melanophores attendant upon variations in temperature are not responsible for the particular manner in which the pulsations of these cells respond to alterations in temperature. Whether viscosity is of importance in governing the non-pulsatory changes which occur in the distribution of melanophore pigment, as a fish adapts itself to shifts in the color of its background, is quite possibly another matter and one about which nothing can be stated here.

Summary

1. Pulsations in isolated scale melanophores of *Fundulus heteroclitus* occur in N/10 NaCl after treatment with N/10 $BaCl_2$ at any temperature between 12° and 32° C.

2. Below 12° C. pulsations cease, the melanophore coming to rest in the maximally expanded state.

3. Above 32° C. pulsations also cease, the melanophore coming to rest in the punctate state.

4. Between 12° and 32° C. the extent of the pulsations decreases in a rectilinear manner with an increase in temperature.

5. Between 12° and 27° C. the rate of pulsations increases in a non-rectilinear manner with an increase in temperature. With a further rise in temperature above 27° C. there is a rapid decrease in the rate of pulsations.

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